Table 7-2
Highly conserved amino acid residues in hemoglobins

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino acid</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8</td>
<td>Histidine</td>
<td>Proximal heme-linked histidine</td>
</tr>
<tr>
<td>E7</td>
<td>Histidine</td>
<td>Distal histidine near the heme</td>
</tr>
<tr>
<td>CD1</td>
<td>Phenylalanine</td>
<td>Heme contact</td>
</tr>
<tr>
<td>F4</td>
<td>Leucine</td>
<td>Heme contact</td>
</tr>
<tr>
<td>B6</td>
<td>Glycine</td>
<td>Allows the close approach of B and E helices</td>
</tr>
<tr>
<td>C2</td>
<td>Proline</td>
<td>Helix termination</td>
</tr>
<tr>
<td>HC2</td>
<td>Tyrosine</td>
<td>Cross-links the H and F helices</td>
</tr>
<tr>
<td>C4</td>
<td>Threonine</td>
<td>Uncertain</td>
</tr>
<tr>
<td>H10</td>
<td>Lysine</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

Figure 7-20
Oxygen dissociation curves of myoglobin and hemoglobin. Saturation of the oxygen-binding sites is plotted as a function of the partial pressure of oxygen surrounding the solution.

Figure 7-21
Hill plot for the binding of O₂ to myoglobin and hemoglobin. The slope of 2.8 for hemoglobin indicates that it binds oxygen cooperatively, in contrast with myoglobin, which has a slope of 1.0.

Figure 7-22
Oxygen dissociation curve of hemoglobin. Typical values for pO₂ in the capillaries of active muscle and in the alveoli of the lung are marked on the horizontal axis. Note that P₅₀ for hemoglobin under physiological conditions lies between these values.
In hemoglobin, acidity enhances the release of oxygen

Also, increasing the concentration of CO₂ lowers the oxygen affinity.

The Bohr effect: The presence of higher levels of CO₂ and H⁺ in the capillaries of metabolically active tissue promotes the release of O₂ from oxyhemoglobin. In the alveolar capillaries of the lungs, the high concentration of O₂ unloads H⁺ and CO₂ from hemoglobin.

The oxygen affinity of hemoglobin within red cells is lower than that of hemoglobin in free solutions.

2,3-bisphosphoglycerate (BPG) binds to hemoglobin and lowers its oxygen affinity.

Fetuses have their own kind of hemoglobin called hemoglobin F (α₂γ₂) [Isoforms or isotypes]

Hemoglobin F has a higher oxygen affinity than does hemoglobin A, due to its lower binding to BPG.
(1) The sequential model makes the following three assumptions:

- only two conformational states, T and R, are accessible to any subunit
- the T to R transition in a subunit is induced by the binding of ligand to that particular subunit
- the conformational change elicited by the binding of substrate in one subunit can increase or decrease the binding affinity of the other subunits in the same molecule.

(2) The concerted model makes the following three assumptions:

- all the subunits of a particular molecule must be in the T form, or all must be in the R form
- ligands bind with low affinity to the T form, and with high affinity to the R form
- the binding of each ligand increases the probability that all subunits in that molecule are in the R form.
Two models offer contrasting views of how cooperative interactions occur in hemoglobin:

(a) Sequential model

\[ \square = T \text{ form} \]
\[ \bigcirc = R \text{ form} \]

Figure 7-37
Simple sequential model for a tetrameric allosteric protein. The binding of a ligand to a subunit changes the conformation of that particular subunit from the T (square) to the R (circle) form. This transition increases the affinity of the other subunits for the ligand.

(b) Concerted model

\[ K_T \]
\[ K_R \]

Figure 7-39
Concerted (Monod-Wyman-Changeux, or MWC) model for a tetrameric allosteric protein. The squares denote the T form, and the circles denote the R form. The ratio of T to R forms in the absence of ligand is L. The dissociation constants for the binding of ligand to the T and R states are \( K_T \) and \( K_R \).
Figure 8-23, page 199

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Impact of the discovery of molecular diseases

Analyses of mutations affecting oxygen transport have had a major impact on molecular biology, medicine, and genetics.

1. They are sources of insight into relations between the structure and function of normal hemoglobin.

2. The discovery of mutant hemoglobin has revealed that disease can arise from a change of a single amino acid in one kind of polypeptide chain.

3. The finding of mutant hemoglobin has enhanced our understanding of evolutionary process.
Sickle-cell anemia

In 1904 a Chicago physician examined a 20 year old black college student who suffered from cough, fever and headache.

- examination of the blood showed that the patient was anemic (↓ # of red blood cells)
- the shape of the red cells was very unusual and irregular (sickle-shaped cells)

- the incidence of sickle-cell anemia among blacks is about 4 per 1000
- it is a genetic disease. Patients with sickle-cell anemia are homozygous for the abnormal gene. Heterozygous people are usually not symptomatic.
- the sickle-cell hemoglobin is referred to as "hemoglobin S"

The frequency of the sickle gene is as high as 40% in certain parts of Africa.

Sickle-cell trait confers a small but highly significant degree of protection against malaria, perhaps by accelerating the destruction of infected erythrocytes.
Sickle-cell anemia

**Figure 7-43**
Net charge of sickle-cell hemoglobin and of normal hemoglobin as a function of pH, as measured by their electrophoretic mobilities. The isoelectric point of a molecule is the pH at which its mobility is 0.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Sickle-cell</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyhemoglobin</td>
<td>6.87</td>
<td>7.09</td>
<td>0.22</td>
</tr>
<tr>
<td>Deoxyhemoglobin</td>
<td>6.68</td>
<td>6.91</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**Figure 7-44**
Gel electrophoresis pattern of hemoglobin isolated from a normal person, from a person with sickle-cell trait, and from a person with sickle-cell anemia.

**Figure 7-45**
Fingerprinting. A mixture of peptides produced by proteolytic cleavage is resolved by electrophoresis in the horizontal direction followed by chromatography in the vertical direction.
Molecular Pathology of hemoglobin

There are more than 300 abnormal hemoglobins that have been discovered. Abnormal hemoglobins are of four types:

(1) Altered exterior

(2) Altered active site

(3) Altered tertiary structure

(4) Altered quaternary structure

Thalassemias are genetic disorders of hemoglobin synthesis. They are produced by many different mutations that lead to the absence or deficiency of a globin chain in a variety of ways.